Mini Prep

1. inoculation

a. touch culture and grow on sterile media

b. if transformation colonies

i. 20 uL of STERILE water, touch colony and mix into sterile water

ii. put 3.5 mL of LB medium into culture tube

iii. put 15 uL of the 20 uL colony solution into the culture tube

iv. put on shaker, keep tube at angle for more aeration

v. for remaining 5ul, go to colony PCR (optional)

2. centrifuge culture (make pellet)

. use 15 mL conical centrifuge tube to not lose cap in centrifuge

a. spin down at (3,000 RPM for 5 mins for a good centrifuge) 5,000 RPM for 7 mins on old BE centrifuge at Room temperature

b. pour off supernatant

3. remove RNA

. take P1 out of fridge, check the cap sticker to make sure RNase is added

a. add 250 uL P1 buffer and resuspend (pipet mix)

b. after using P1, keep it at 4C in fridge

c. transfer to new centrifuge tube 1.5 mL (LABEL!)

4. lyse cells with detergent

. add 250 uL P2 buffer

a. invert 4-6 times to mix

b. CHECK:

. DO NOT leave lysis buffer in pellet for more than 5 mins

i. viscous

5. ;precipitate unwanted content with neutralization buffer (heavy genome sinks to bottom, plasmid stays on top)

. add 350 uL N3 buffer

a. invert 4-6 times ***vigorously***

b. centrifuge 13,000 RPM for 10 mins

c. remove tube from centrifuge gently!!, carry rack over

6. filter supernatant to purify plasmid

. remove 900 uL supernatant onto column

a. Be careful not to get ANY of the white pellet

b. centrifuge 13,000 RPM for 1 min

c. pour off flow-through in liquid waste

7. wash with alcohol PE buffer to keep plasmid stuck on filter

. check the cap sticker to make sure ethanol is added

a. add 750 uL PE buffer to column filter

b. centrifuge 13,000 RPM for 1 min, no liquid should be left on filter

c. pour off Ethanol flow-through in collection tube into liquid waste

8. evaporate alcohol by spin

. centrifuge 13,000 RPM for 1 min

9. add Elution Buffer (EB: H20+ salts) to filter to elute DNA

. <u>move filter over brand new Eppendorf 1.5mL tube</u>

a. add 50 uL EB to filter

b. Let SIT ON BENCH on filter for 1 min

c. centrifuge 13,000 RPM for 1 min

10. Nanodrop measures DNA concentration at 260nm and 280nm (gives ratio close to 1.8-2.0)

. 1.5 uL

a. wipe and dry cover and sensor with H2O

b. it will ask you what kind of sample? click nucleic acid

c. click no for the next question and make sure the arm is down

d. Load a blank: EB buffer (1.5 microliters) and small tips

e. load it right onto the dot and gently close the lid, click play

f. Hit blank

g. how will you know it blanked correctly? clean it off and add another drop, treat is as your first sample, it should be a flat line within accuracy of 2

h. add actual sample now and record concentration